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Pulsing of Dendritic Cells With Cell Lysates From Either B16 Melanoma or MCA-106 Fibrosarcoma Yields Equally Effective Vaccines Against B16 Tumors in Mice

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Background and Objectives: Dendritic cells (DC) pulsed in vitro with a variety of antigens have proved effective in producing specific antitumor effects in vivo. Experimental evidence from other laboratories has confirmed that shared antigens can be encountered in histologically distinct tumors. In our experiments, we set out to evaluate the immunotherapeutic potential of vaccines consisting of DC pulsed with MCA-106 fibrosarcoma or B16 melanoma cell lysates and to determine whether a cross-reactivity exists between the two tumors.

Methods: DC were prepared from the bone marrow of C57BL/6 (B6) mice by culturing progenitor cells in murine granulocyte-macrophage colony-stimulating factor (GM-CSF). They were separated into three equal groups and were either pulsed with B16 melanoma cell lysates (BDC), pulsed with tumor extract from the syngeneic fibrosarcoma MCA-106 (MDC), or left unpulsed (UDC). DC were then used to immunize three groups of mice, with all mice receiving two weekly intravenous (IV) doses of 1×10^6 DC from their respective preparations on days −14 and −7. A fourth group of control mice were left untreated. On day 0, all mice were challenged with subcutaneous injections of 1×10^5 B16 and 1×10^5 MCA tumor cells, administered in the left and right thighs, respectively. After the inoculations, the mice were monitored closely with respect to tumor growth and survival.

Results: The MDC mice developed specific cellular immunity directed against not only MCA-106 tumor cells, but also against B16 melanoma, as measured through chromium-release assays of splenocyte preparations, while remaining ineffective at killing both L929 fibroblasts and CT26 tumor cells. By day 30 after tumor inoculations, control mice manifested the largest B16 tumor volumes at a mean of 2185 mm^3, followed by the UDC, MDC, and BDC groups at 92 mm^3 ($P = 0.00008$), 3 mm^3 ($P = 0.000002$), and 2 mm^3 ($P = 0.00004$), respectively. The survival data

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mirrored this pattern, with control animals displaying the shortest mean survival time (37.1 ± 4.0 days), followed by UDC (44.8 ± 6.6), MDC (56.2 ± 14.7), and BDC (56.4 ± 18.3) animals. No significant differences were noted between MCA-106 and B16 cell lysate-pulsed DC vaccines with respect to their abilities to inhibit B16 tumor growth and to prolong survival. These findings were confirmed using a B16 pulmonary metastasis model. Likewise, vaccination with interferon-γ gene-modified MCA-106 tumor cells was shown to be effective at protecting against a subsequent subcutaneous B16 tumor challenge in 3 of 4 mice observed.

Conclusions: These results demonstrate that immunization with antigen-pulsed DC confers cellular immunity, retards tumor growth, and prolongs the survival of tumor-challenged mice. The ability of MCA-106 cell lysate-pulsed DC vaccines to inhibit the growth of subcutaneous B16 tumors also suggests the presence of shared tumor-associated antigens between these two histologically distinct tumors.

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KEY WORDS: dendritic cells; tumor lysates; MCA-106 fibrosarcoma; B16 melanoma; cross-reactivity; vaccine

INTRODUCTION

Dendritic cells (DC) are specialized antigen-presenting cells (APC) found in small numbers in various tissues throughout the body [1–3]. They are strategically located at sites of antigen exposure [4] and have the ability to process tumor antigens and express them in conjunction with the major histocompatibility complex (MHC) class I and II molecules. Within the local lymphatics, they present these antigens to naive $CD4^+$ [5–8] and $CD8^+$ [9,10] T lymphocytes, and to B lymphocytes, inducing T- and B-cell immunity [11].

Cytotoxic T lymphocytes (CTLs) are central players in the host immune response against tumors [12]. DC are extremely effective at the task of T-cell activation [13–16] by virtue of cell surface adhesion and co-stimulatory molecules such as ICAM-1, LFA-3, B7.1 (CD80), and B7.2 (CD86) [17], which they express, and through the production of cytokines [18–21]. DC pulsed with antigenic proteins [22,23], peptides [10,24–26], tumor cell fragments [27,28], or tumor RNA [29] in vitro have demonstrated the ability to stimulate specific CTL reactions and to establish tumor immunity when administered in vivo.

DC clearly have an essential role in immunosurveillance and in stimulation of antitumor immunity, capacities that make them ideal effectors to be incorporated in immunotherapeutic vaccines against cancer [30,31]. In the past, the inability to generate large numbers of DC was a limiting factor against their use in vaccines. Recently developed protocols, however, have allowed the generation of larger numbers of DC from murine bone marrow [32] and have made it easier to further examine the therapeutic potential of DC. Likewise, new techniques to isolate significant numbers of DC from human bone marrow [33] and peripheral blood [34–36] have rendered their use in human vaccines more realistic. In a recent human study involving B-cell lymphoma patients, vaccination with antigen-pulsed DC resulted in measurable tumor regressions and decreased development of new tumors [37].

The discovery of tumor antigens recognized by T cells in mice [38,39] revolutionized the field of tumor immunology. Since then, multiple tumor-associated antigens, usually associated with the MHC class I molecule, have been described for human melanoma [40–44], opening the door for specific active immunotherapy trials using these antigens as immunogens. Unfortunately, the search for such antigens for other solid tumors has been unsuccessful in most cases. The MAGE [45,46], BAGE [47], and GAGE [48] antigens are expressed in normal testis tissue, as well as in melanoma and other tumors [49]. Along with other markers and oncogenes shared by dissimilar malignancies, these provide evidence of common traits among neoplasms. It is hoped that these shared antigens will be exploited in the future to combat less immunogenic tumors that have proved resistant to conventional therapies.

It has been shown in mice that the immunogenic fibrosarcoma MCA-105 shares common tumor-associated peptide epitopes with subclones of the unrelated, relatively nonimmunogenic B16/BL6 melanoma [50]. In this work, we demonstrated that vaccination with DC pulsed with cell lysates obtained from another 3-methylcholanthrene-induced murine fibrosarcoma, MCA-106, elicits specific cellular immunity directed against the histologically distinct B16 melanoma, inhibits the growth of B16 tumors in vivo and improves survival in tumor-bearing mice.

MATERIALS AND METHODS

Animals

The 5 to 6 week-old female C57BL/6 (B6,H-2^b) mice used in these experiments were obtained from Charles River Laboratories (Raleigh, NC). The animals were housed at the Durham Veteran's Administration Medical Center (DVAMC) animal facilities (Durham, NC). Basic care was provided by the staff at the DVAMC animal facilities. All animal studies were performed in accordance with the animal care policy of the Duke University Animal Care and Use Committee. Both the animal facilities and Duke University are approved by the American Association for Accreditation of Laboratory Animal Care (AALAC).

Preparation of Dendritic Cells From Murine Bone Marrow

Dendritic cells were prepared from the bone marrow of C57BL/6 mice according to a protocol previously described by other investigators [32,51]. Briefly, the bone marrow was flushed from the long bones of the hindlimbs and depleted of red blood cells by incubating in 0.84% ammonium chloride for 10 min at 37°C. The sample was washed twice in Hank's balanced salt solution (HBSS). Bone marrow cells were then depleted of B cells, T cells, and I-A^+ cells by incubating the specimen with anti-B220 (Ra3–3A1/6.1, TIB 146, American Type Culture Collection [ATCC], Rockville, MD), anti-CD8 (2.43, TIB 210, ATCC), anti-CD4 (GK1.5, TIB 207, ATCC), and anti-I-A^b (25–5–165, HB37, ATCC) antibodies in the presence of rabbit complement (1:15). Incubation was performed at 37°C for 1 hr, with frequent shaking. The cells were again washed three times in HBSS. The final pellet was resuspended in DC medium consisting of RPMI supplemented with 10% fetal bovine serum (FBS) (Live Technologies, Gibco Laboratories, Grand Island, NY), 2 mM glutamine, 2β-mercaptoethanol (2×10^{-5} M), penicillin (100 U/ml), streptomycin (100 μg/ml), gentamicin (50 μg/ml), and murine granulocyte-macrophage colony-stimulating factor (GM-CSF) (3 ng/ml) and 10% culture supernatant from L929 fibroblasts. The cells were plated in 6-well culture plates (Becton Dickinson, Franklin Lakes, NJ) at 3×10^6 cells/well. After 3 days of culture, floating cells were gently aspirated, and fresh medium was added to the plate. After 5–7 days, aggregates of nonadherent DC appeared. Their identity was confirmed by fluorescence-activated cell sorter (FACS) analysis.

Cell Surface Antigen Analysis by Indirect Immunofluorescence

Cultured DC were phenotyped by indirect immunofluorescence using the monoclonal antibodies 33D1 (anti-DC, Tib 227, ATCC), F4/80 (anti-macrophage, HB198, ATCC), anti-I-A^b (25–5–165, HB37, ATCC), anti-B220 (Ra3-3A1/6.1, TIB146, ATCC), and anti-Lyt2.2 (TIB 210, ATCC). Cultured B16 and MCA-106 tumor cells were phenotypes using anti-H-$2K^b$ (28–13–35, ATCC), anti-H-$2D^b$ (28–14–8, ATCC), and anti-I-A^b antibodies, in order to quantify the expression of MHC antigens on their cell surface. The assays were performed as described previously [52], using the primary antibodies, followed by a fluorescein isothiocyanate-labeled Fab'_2 anti-mouse secondary antibody (TAGO Immunologicals; Biosource International, Camarillo, CA). Cells were examined for percent positivity and mean channel number (MCN) by FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA).

Preparation of Cell Lysates for DC Pulsing

MCA-106 fibrosarcoma and B16 melanoma cell lysates were prepared from cultured cells according to the protocol previously described by Grabbe et al. [28]. Tumor cells were removed from the substratum using trypsin, washed twice in HBSS, and resuspended at 1×10^7 cells/ml in serum-free AIM V media (Gibco). The cells were treated with four freeze/thaw cycles (alternating dry ice/37°C water bath), and the product was centrifuged at 1,500*g* for 5 min. The supernatant was then collected and centrifuged at 13,000*g* for 60 min. The final supernatant was filtered through a 0.2-μm filter and aliquoted into freezing vials to be stored at −70°C until needed.

Immunization of Mice Using Tumor Cell Lysate-Pulsed DC Vaccine

After 5–6 days of culturing, DC were collected from the 6-well plate, washed in HBSS, and counted. Approximately 4×10^7 DC were obtained from the long hind bones (femur and tibia) of 10 mice. These DC were resuspended in 6 ml DC medium and separated into three groups. A total of 2 ml of MCA-106 and B16 cell lysates (product of 2×10^7 cells) was added to the MDC and BDC, respectively. The UDC were left unpulsed. All three specimens were incubated overnight at 37°C. The next morning, the DC were collected, washed twice in HBSS, and resuspended in 5 ml HBSS-2% human serum albumin (HSA) for injection. Mice from the respective treatment groups were immunized with this preparation, with each mouse receiving two intravenous doses of 1×10^6 pulsed DC (0.5 ml) separated by a 1-week interval. The entire process was repeated 7 days later, for a total of two immunizations per treated mouse. Naive mice, sequestered to serve as a control, were left untreated.

Preparation of Splenocytes for Cytotoxicity Assays

Seven days after the second vaccination, representative mice were selected at random from each group and sacrificed. Their spleens were isolated, placed in HBSS, and pressed into a Petri dish through a sterile steel wire

mesh. The splenocytes were depleted of red blood cells through incubation in 0.84% ammonium chloride for 10 min at 37°C. The sample was washed twice in HBSS. The cells were resuspended in culture medium consisting of RPMI supplemented with 10% FBS, 2β-mercaptoethanol, and 10 U/ml interleukin-2 (IL-2) (kindly provided by Chiron Viagene, San Diego, CA). Splenocytes were stimulated with irradiated MCA-106 or B16 tumor cells (10,000 rads) at a 1:100 (tumor cell to spleen cell) ratio. They were then cultured for 5 days in IL-2-containing media before being used in the cytotoxicity assays.

Cytotoxicity Assays

CTLs from each respective culture were tested. Cell-mediated lysis was determined in vitro using a standard chromium (^{51}Cr)-release assay. Target cells were isolated from a monolayer. Approximately 1–5 × 10^6 target cells were suspended in culture medium and labeled with 75 μCi of ^{51}Cr (New England Nuclear, Boston, MA) for 1 hr at 37°C, then washed three times in HBSS. They were resuspended in culture medium at 5 × 10^4 cells per milliliter and combined in triplicate wells of round-bottomed microtiter plates (Serocluster, Costar, Cambridge, MA) with varying numbers of effector cells in order to obtain effector-to-target ratios of 100:1, 50:1, and 25:1. The final volume of each well was adjusted to 200 μl using RPMI-10% FBS medium. Wells which contained target cells only with either culture medium or 1 M HCl served as spontaneous and maximal release controls, respectively. The plates were centrifuged for 5 min at 200*g* and incubated at 37°C in 5% CO_2 for 6 hr. The supernatants were harvested using a harvesting frame (Skatron Harvesting System, Skatron Sterling, VA) and were counted in a gamma counter. The cytotoxic index was calculated using the measured counts per minute (cpm) in the following equation:

$$\frac{\text{Mean of experimental lysis} - \text{spontaneous release}}{\text{mean of maximal release} - \text{spontaneous release}} \times 100\%.$$

Preparation of Tumor Cells for in Vivo Experiments

MCA-106 fibrosarcoma and B16 melanoma were used in all in vivo experiments. MCA-106 and B16 cells were obtained from subcutaneous nodules previously established in C57BL/6 mice. The tumor nodules were harvested from the mice under sterile conditions. The specimen was mechanically dispersed in HBSS, then digested with a mixture of deoxyribonuclease (10 μg/ml), collagenase (1 mg/ml), and hyaluronidase (2.5 U/ml) for 15–30 min, in order to obtain a single cell suspension. Most of the specimen was cryopreserved for future use. Small volumes of each tumor were grown as monolayers in T75 flasks (Corning, Cambridge, MA) and maintained in Eagle's minimum essential medium (MEM) (Gibco) supplemented with 5% FBS. Monolayers were propagated by trypsinization as required. Cells were kept at 37°C in a humidified atmosphere containing 5% CO_2. The day the subcutaneous tumors were established, the cells were dislodged from the substratum using trypsin, washed twice in HBSS, counted, and resuspended in HBSS-2% HSA at a concentration of 4 × 10^5 cells/ml. This preparation was then used immediately to induce subcutaneous tumors.

Establishment of Subcutaneous Tumors and Follow-up

Seven days after the treated groups had been vaccinated with DC for the second and final time, all mice received inocula of 1 × 10^5 MCA–106 and 1 × 10^5 B16 tumor cells delivered subcutaneously in the right and left thighs, respectively. The animals were then monitored closely until the first palpable tumors appeared, at which time the first three-dimensional tumor measurements were obtained, using calipers. Thereafter, tumor measurements and survival data were obtained every 2–3 days and recorded. These experiments were performed with reproducible outcomes. The data, as they are presented, represent a compilation of results.

B16 Pulmonary Metastases Experiment

Mice were separated into three groups of 6, i.e., control, MCA-pulsed DC, and B16-pulsed DC groups. Both DC vaccine groups received two weekly dose of 1 × 10^6 of their respective DC preparations intravenously (IV), while control mice were left untreated. Seven days after the final immunizations, all mice were challenged with 1 × 10^5 B16 tumor cells IV, in order to establish pulmonary metastases. Animals were followed closely during the ensuing weeks. Determination of pulmonary metastatic burden was performed at the time of death for animals that died before day 35. All other mice were euthanized on day 35 and the number of B16 metastases were counted under a dissecting microscope. The converse experiment using a model of MCA-106 pulmonary metastases was not performed, because of the inability of MCA-106 cells to proliferate in pulmonary tissue.

Immunization of the Mice Using IFN-γ-Transduced Tumor Cells

Previous work in our laboratory has shown that IFN-γ-transduced MCA-106 and B16 tumor cells are capable of inducing specific cellular immunity and protecting mice against a tumor challenge [53]. In this study, we used immunization with IFN-γ-transduced tumor cells as a second system to determine if a cross-reactivity exists between MCA-106 and B16 tumor cells. Transduction of

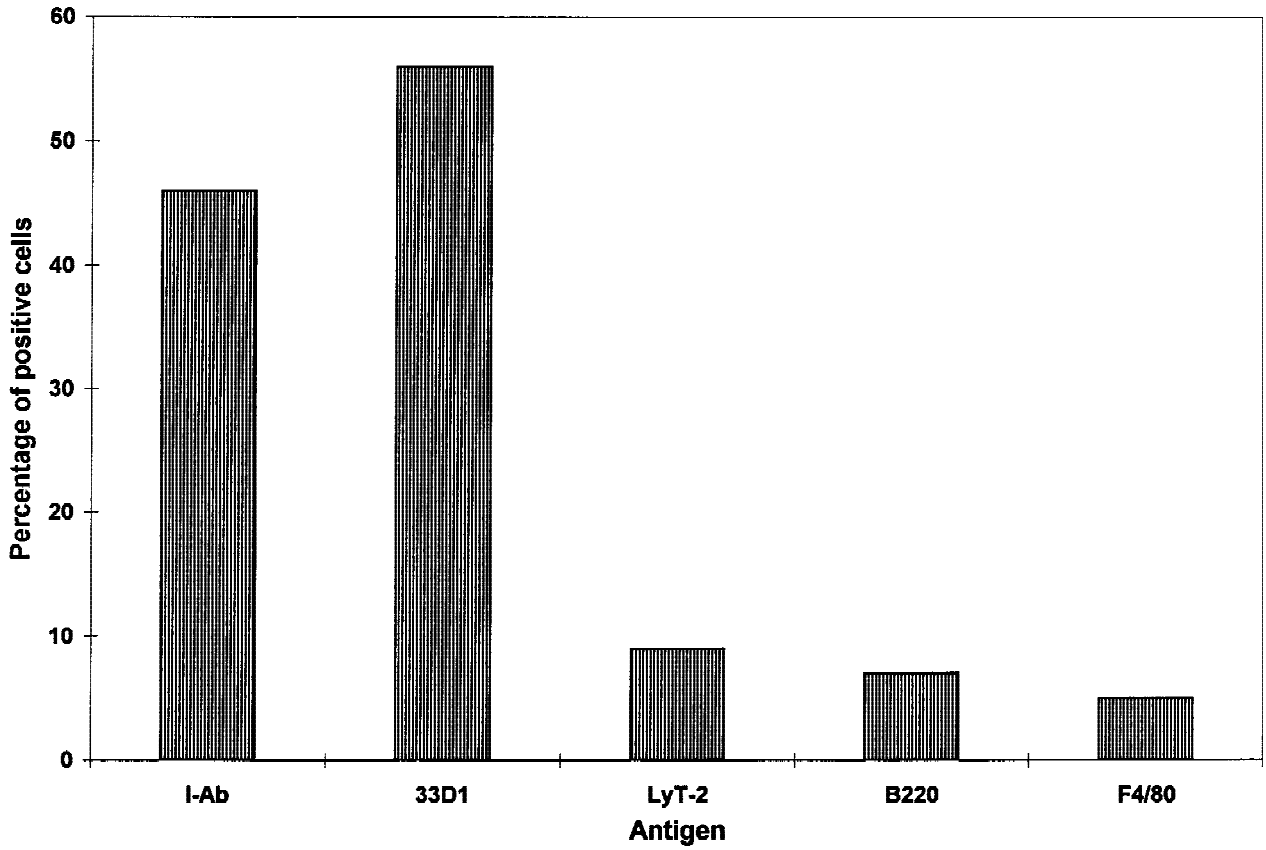

Fig. 1. Flow cytometric analysis of murine bone marrow-derived dendritic cells (DC). Note the high levels of DC markers (I-A^b, 33D1) with concomitant low expression of T cell (LyT-2, 9%), B-cell (B220, 7%), and macrophage (F4/80, 5%) antigens.

MCA-106 and B16 tumor cells was performed using a retroviral vector construct carrying the gene for murine IFN-γ, as previously described [53]. Mice from two respective groups were then immunized with three intraperitoneal injections of either 1×10^6 IFN-γ-transduced MCA-106 (MCA-γ) or 1×10^6 IFN-γ-transduced B16 (B16-γ) tumor cells, administered on days −21, −14, and −7. Subcutaneous tumors were then established on day 0 using parental tumor cells. All mice were injected in the right thigh with 1×10^5 cells from the tumor line against which they had been vaccinated (e.g., B16-γ mice were inoculated with B16 tumor cells). Tumor inoculations were also given in the left thigh, with one-half of the mice in each group getting 1×10^5 tumor cells from the tumor line against which they had not been vaccinated (i.e., B16-γ mice were injected with MCA-106 tumor cells and vice versa) and the other half receiving 1×10^5 Lewis lung tumor (LLT, nonsyngeneic) cells. The mice were then followed for the development of subcutaneous tumors and tumor measurements were recorded.

Statistical Analyses

Student's *t*-test was used to evaluate the significance of differences between experimental groups. A *P*-value of <0.05 was considered significant. Reported *P*-values in the DC/subcutaneous tumor experiments are in comparison with the control group, unless otherwise specified.

RESULTS

Generation of DC From Murine Bone Marrow

DC were successfully generated from the bone marrow of C57BL/6 mice. FACS analysis confirmed their phenotype, with high expression of MHC class II molecules (I-A^b) and DC markers (33D1), at 46% and 56% of the cells assayed, respectively. Meanwhile, very few of these cells manifested either T-cell, B-cell, or macrophage antigens (Fig. 1).

Expression of MHC Surface Antigens in B16 and MCA-106 Tumor Cells

B16 tumor cells expressed low levels of both MHC class I and II molecules. Although MCA-106 tumor cells also manifested low levels of MHC class II antigens, their expression of class I molecules was significantly elevated as compared with B16 (Table I).

TABLE I. Expression of MHC Surface Antigens in B16 and MCA-106 Tumor Cell Lines*

Cells	K^b (%)	D^b (%)	I-A^b (%)
B16	4	5	5
MCA-106	63	97	2

*MHC, major histocompatibility complex.

Vaccination With MCA-106 Cell Lysate-Pulsed DC Elicited Cellular Immunity Against Both B16 and MCA-106 Tumor Cells, as Detected Through in Vitro Cytotoxicity Assays

After 5 days of in vitro culturing of splenocytes in IL-2-containing media, cytolytic activity was measured through ^{51}Cr-release assays. When compared to those isolated from UDC and control mice, splenocytes from MDC mice clearly demonstrated increased in vitro cytotoxicity directed not only against MCA-106 tumor cells, but also against B16 cells (Fig. 2). L929 and CT26 cells were not killed at any E:T ratio, underlining the specificity of the cellular immunity.

B16 and MCA-106 Cell Lysate-Pulsed DC Vaccines Were Equally Effective at Suppressing the Growth of Subcutaneous B16 Tumors in Vivo

Control mice manifested the first evidence of both B16 and MCA-106 tumor growth. By day 21, all control mice (12/12) had developed grossly measurable B16 tumors, in contrast to only 25% of the UDC (3/12), 17% of the MDC (2/12), and 8% of the BDC (1/12) groups. At the same point in time, all untreated mice were also afflicted with palpable MCA-106 tumors, while only 67% (8/12), 50% (6/12), and 50% (6/12) of the UDC, BDC, and MDC mice, respectively, were affected. Comparison of tumor burdens was performed on day 30 after tumor inoculation, at a time when all mice in each group were still alive. The three-dimensional tumor measurements were used to calculate a diameter for each tumor. Using this value, tumor volumes were then calculated using the formula

$$\text{Vol} = 4/3\ \pi r^3.$$

As expected, the control group had the largest tumors bilaterally, with mean B16 and MCA-106 tumor volumes of 2,185 mm^3 and 776 mm^3, respectively. The UDC mice displayed intermediate-size tumors, with a B16 volume of 92 mm^3 ($P = 0.00008$) and an MCA-106 volume of 478 mm^3 ($P = 0.5$). Meanwhile, BDC and MDC mice manifested the smallest tumors, with very similar mean B16 tumor volumes of 2 mm^3 ($P = 0.00004$) for the former and 3 mm^3 ($P = 0.000002$) for the latter. The converse was not as striking, but nonetheless significant, as BDC and MDC mice displayed MCA-106 tumors with mean volumes of 97 mm^3 ($P = 0.02$) and 28 mm^3 ($P = 0.03$), respectively (Table II).

B16 and MCA-106 Cell Lysate-Pulsed DC Vaccines Were Equally Effective at Prolonging Survival

Control animals were the first to succumb to tumor burden. The first control animal died 30 days after subcutaneous tumor inoculation and all twelve were dead by day 44, at which time 50% (6/12) of the UDC, 83.3% (10/12) of the BDC, and 100% (12/12) of the MDC mice remained alive. The last of the UDC mice died on day 53, with 5 mice (41.7%) still remaining in each of the pulsed DC vaccine groups. The BDC and MDC curves paralleled each other closely, with the last animal in either group dying on day 104 after tumor inoculations (Fig. 3). Mean survival was only 37.1 ± 4.0 days for the controls, while the UDC, BDC, and MDC mice survived 44.8 ± 6.6 ($P = 0.002$), 56.4 ± 18.3 ($P = 0.002$), and 56.2 ± 14.7 ($P = 0.0003$) days, respectively. Survival times for the BDC and MDC groups were significantly different from those of the UDC group, with *P*-values of 0.05 (UDC vs. BDC) and 0.02 (UDC vs. MDC).

MCA-106 Lysate-Pulsed DC Vaccines Were Effective at Suppressing the Growth of B16 Pulmonary Metastases

Within 35 days, all 6 control mice had died with heavy pulmonary tumor burden, i.e., almost complete pulmonary parenchymal replacement by tumor, while all treated mice survived. On day 35, treated animals were euthanized, and their lungs were excised to assess tumor burden. The mean number of B16 pulmonary metastases encountered was 31.7 ± 26.7 (range 2–82) for the MCA-106 lysate-pulsed group and 62.3 ± 27.4 (range 15–99) for B16 lysate-pulsed mice ($P = 0.08$).

Vaccination With IFN-γ Gene-Modified MCA-106 Tumor Cells Was Effective in Protecting Against a Subsequent B16 Tumor Challenge

LLT tumors developed in all inoculated animals from both the B16-γ (6/6) and MCA-γ (4/4) groups. Uniformly progressive tumor growth was seen in each group, with mean LLT tumor volumes of 6,935 mm^3 for the former and 7,274 mm^3 for the latter, as measured on day 30 after tumor inoculation. Meanwhile, the mice were successful at fending off the tumors against which they had been immunized, with only 13% of MCA-γ mice (1/8, mean tumor volume of 14.1 mm^3) developing MCA-106 tumors and 33% of B16-γ mice (4/12, mean tumor volume of 25.7 mm^3) manifesting B16 tumors.

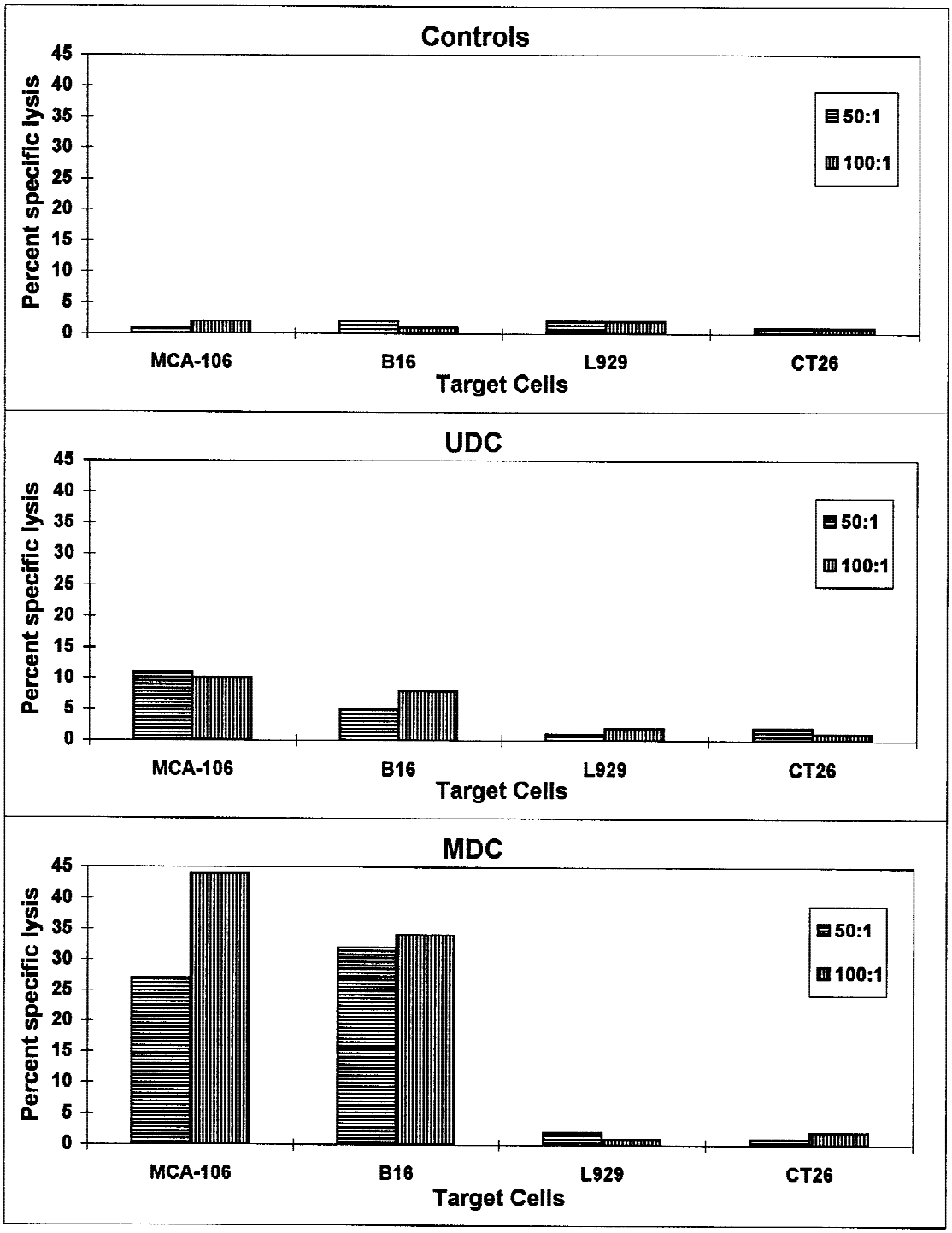

Fig. 2. Cytotoxicity profiles for the control, UDC, and MDC groups as measured through ^{51}Cr-release assays at 50:1 and 100:1 effector to target ratios. Note that splenocytes isolated from MDC mice display cellular immunity against both MCA-106 and B16 tumor cells. UDC, mice immunized with unpulsed dendritic cells; MDC, mice immunized with MCA-106 cell lysate-pulsed dendritic cells.

While B16-γ mice were incapable of defending themselves against MCA-106 (6/6 with tumor, mean volume of 2,193 mm^3), only 25% of the MCA-γ mice developed a B16 tumor (1/4, mean tumor volume of 5 mm^3) (Figure 4, Table III).

DISCUSSION

In this study, we have demonstrated that

1. Vaccination with MCA-106 cell lysate-pulsed DC can:

TABLE II. Mean Tumor Volumes by Treatment Groups (d30)

Group	B16 (mm^3)	*P**	MCA-106 (mm^3)	*P**
Control	2,185	—	776	—
UDC[a]	92	0.00008	478	0.5
BDC[b]	2	0.00004	97	0.02
MDC[c]	3	0.000002	28	0.03

*Reported *P*-values in comparison with the control group.
[a]UDC, mice immunized with unpulsed DC.
[b]BDC, mice immunized with B16 cell lysate-pulsed DC.
[c]MDC, mice immunized with MCA-106 cell lysate-pulsed DC.

a. Elicit significant cellular immunity directed against B16 melanoma, as confirmed through in vitro cytotoxicity assays;
b. Inhibit the growth of subcutaneous B16 tumors;
c. Impede the establishment of B16 pulmonary metastases;
d. Prolong the survival of MCA-106 and B16 tumor-bearing mice.

2. Vaccination with B16 cell lysate-pulsed DC can:
   a. Interfere with the development of subcutaneous MCA-106 tumors;
   b. Improve the survival of MCA-106 and B16 tumor-bearing mice.
3. Immunization with IFN-γ-transduced MCA-106 cells is effective in inhibiting the growth of subcutaneous B16 tumors.

In recent years, DC have been shown to play an important role in the rejection of tumors by the immune system. Their infiltration of tumors has been associated with an improved prognosis for many neoplasms, including some originating from the larynx [54], nasopharynx [55], esophagus [56], stomach [57,58], lung [59], prostate [60], and cervix [61]. Furthermore, clinical trials have demonstrated the ability of antigen-pulsed DC to induce tumor regression and inhibit further development of tumors in patients with B-cell lymphoma [37].

Some tumor markers and oncogenes have been found to be present in otherwise distinct tumors. The MAGE-1,-3, BAGE, and GAGE-1,-2 antigens, for example, are all nonmutated proteins expressed in normal testis tissue, as well as in melanoma, non-small cell lung, head and neck, bladder, and breast cancers [49]. Additionally, a small percentage of sarcomas and prostate carcinomas have been shown to express MAGE-1,-3 and GAGE-1,-2 [49]. Similarly, specific peptides derived from the proto-oncogene HER2/neu have recently been associated with ovarian, breast, and non-small cell lung cancers [62–64]. Finally, the cross-reactivity that exists between melanoma and other unrelated tumors of neural crest origin suggests the presence of additional unrecognized shared T-cell-defined epitopes [65].

If oncogenes and their products are included, nearly all solid human malignancies have been linked to tumor-associated antigens. The search for unique, distinct tumor markers, however, has proved fruitful for only a few malignancies. In fact, for most human tumors, specific associated antigens have yet to be discovered. For these neoplasms, immunotherapeutic regimens cannot be based on immunization against defined antigenic peptide epitopes, but must rather employ more general approaches, such as vaccination using irradiated tumor cells or treatment with APCs primed with either cell lysates, acid-eluted peptides, or nucleic acids obtained from the tumors themselves.

B16 melanoma, which arose spontaneously in C57BL/6 mice [66], displays low levels of MHC class I molecules [67] and is poorly immunogenic as a result [68,69], a phenomenon that is probably due to an inability to present tumor antigens at a level sufficient to trigger a TCR signal. Indeed, its low immunogenicity can not be attributed to ineffective tumor antigens, as APCs pulsed with B16-derived peptide epitopes are capable of eliciting specific CTL reactions directed against B16 both in vitro [70] and in vivo [71]. Furthermore, the transfection of B16 with the K^b molecule converts it into a strongly immunogenic tumor in vivo [72] and renders it susceptible to rejection by immunocompetent mice [50]. Likewise, it has recently been demonstrated that stably transfected B16 clones expressing the heat shock protein Hsp65, a ''molecular chaperone,'' manifest significantly elevated levels of the MHC class I molecule and are more easily lysed by alloreactive CTLs [73]. The recent identification of tyrosinase-related protein 2 (TRP-2), a normal tissue differentiation antigen, through screening of a B16 cDNA library with tumor-reactive CTLs [74], came as final evidence. Subsequent in vitro stimulation using TRP-$2_{181–188}$, the major reactive peptide epitope within TRP-2, produced CTLs that were capable of recognizing and reacting against established B16 tumors [74]. Many human tumors display similar deficiencies in endogenous antigen presentation [75,76] that interfere with their ability to elicit appropriate antitumor responses.

The 3-methylcholanthrene-induced fibrosarcomas of C57BL/6 origin, MCA-105 and -106, express the class I molecule and are generally regarded as immunogenic tumors [77]. Although these two murine fibrosarcomas manifest distinct tumor-specific antigens [78,79], TILs isolated from each of the tumor lines, when restimulated in vitro with syngeneic tumor cells in the presence of IL-2, have demonstrated in vivo cross-reactivity with both [80,81]. Similar cross-reactions were demonstrated between MCA-105 and CL8-1, an H-$2K^{b+}$ clone of the BL6-8 melanoma (a subclone of B16), as TILs isolated from MCA-105 lesions recognized not only MCA-105, but also CL8-1, tumor cells, leading to the identification

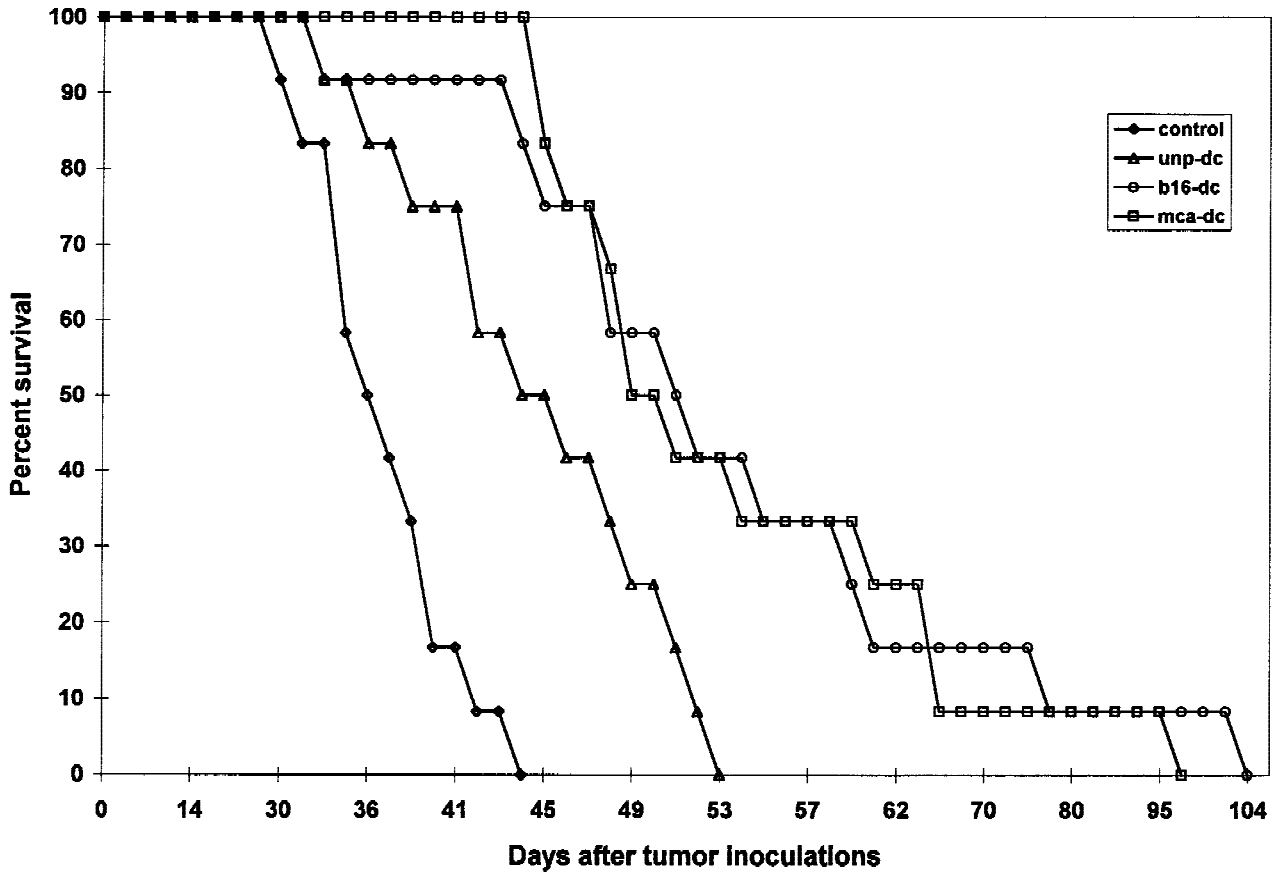

Fig. 3. Survival by treatment group for subcutaneous tumor experiments.

of common H-2K^b-associated peptide epitopes between the two unrelated tumor cell lines [50].

Our experiments have shown that DC pulsed with MCA-106 tumor cell lysates are capable of eliciting anti-tumor reactions in vivo not only against MCA-106, but also against B16 melanoma, suggesting the presence of shared antigens between these two tumors. Likewise, immunization with IFN-γ-modified MCA-106 tumor cells was effective in protecting mice against a subsequent subcutaneous B16 tumor challenge. It is highly unlikely that nonspecific responses are responsible for this cross-protection, as immunization against MCA-106 produced CTLs that displayed minimal lysis of the L929 or syngeneic CT26 cell lines in vitro and no suppression of the LLT tumor in vivo.

Whereas the B16-pulsed DC vaccine was moderately successful in defending against subsequent MCA-106 tumor inoculations, immunization with γ-modified-B16 tumor cells was completely ineffective, with all treated mice developing large subcutaneous MCA-106 tumors. Our γ-modified MCA-106 cell line has previously been shown to produce much more IFN-γ (2,000 U/10^6 cells/day) [82] than our γ-modified B16 cell line (843 U/10^6 cells/day) [83] in quantitative assays of cytokine production, which could partly explain these findings.

The discovery of a variety of tumor-associated antigens in recent years has opened the door for novel anti-tumor therapies using immunologic approaches that target specific peptide epitopes. It is uncertain, however, whether these newly identified antigens are relevant in eliciting anti-tumor reactions in vivo. Indeed, in a recent publication by Anichini et al. [84], most CTLs isolated from HLA-A2.1 melanoma patients did not recognize gp100, Melan/Mart, tyrosinase, or MAGE-3. For the time being, this supports the use of vaccines consisting of APCs pulsed with unfractionated tumor peptides in immunotherapeutic protocols [85], as these are more likely to immunize against relevant tumor antigens and less likely to lead to the formation of escape mutants. These preparations are not free of shortcomings, however, as they immunize against both tumor and self antigens and can conceivably produce autoimmune reactions [84].

In a promising work recently published by Rosenberg et al. [86], a synthetic peptide derived from gp100 and modified to improve binding to HLA-A2 molecules was used as a cancer vaccine to treat patients with metastatic melanoma, yielding successful immunization 91% of the time. Objective clinical responses were observed in 42% of the patients who additionally received IL-2. Future efforts will hopefully uncover the antigens and mecha-

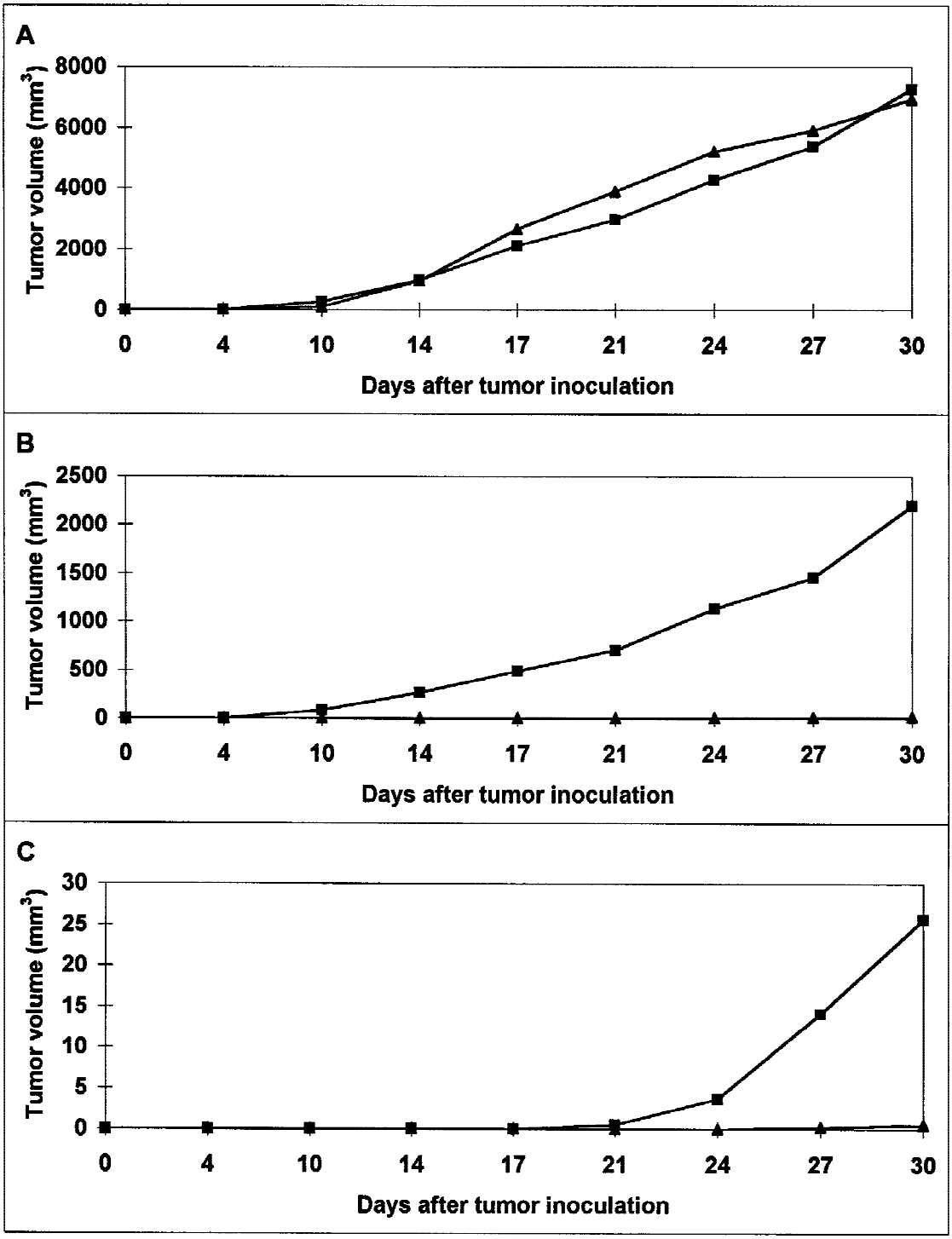

Fig. 4. Mean tumor volumes, as measured at various time intervals after tumor inoculation, for the IFN-γ-modified tumor cell immunization studies. Squares represent mice immunized with B16-γ and triangles those that received MCA-γ. Mean volume curves are plotted for (**A**) LLT (Lewis lung tumor); (**B**) MCA-106; and (**C**) B16 tumors. Immunization with IFN-γ-modified MCA-106 cells was successful in reducing the growth of both MCA-106 and B16 tumors, while vaccination with IFN-γ-modified B16 cells was only capable of reducing the proliferation of B16 tumors. Note that neither immunization protocol was successful in inhibiting growth of the nonsyngeneic LLT tumor.

TABLE III. Mean Tumor Volumes (in mm^3) (d30) for γ-Modified Tumor Cell Experiment

Tumor	MCA-γ	n	B16-γ	n	*P**
B16	0.5	4	25.7	12	0.25
MCA-106	14.1	8	2,193.3	6	0.000085
LLT	6,934.9	4	7,274.5	6	0.61

*Reported *P*-values compare measured tumor volume differences between the MCA-γ and B16-γ groups for each of the respective tumors.

nisms most responsible for immunologic tumor rejection for all cancers, obviating the need to immunize patients with nonspecific preparations.

The data presented herein support the presence of common murine T-cell-recognized tumor associated epitopes in the histologically distinct tumors: melanoma and sarcoma. They also emphasize the effectiveness of DC in processing and presenting tumor-associated antigens, and in producing anti-tumor responses, even in the setting of poorly immunogenic tumors.

CONCLUSIONS

Antigen-pulsed DC have been shown to be effective in producing tumor rejection in vivo, even in the setting of otherwise nonimmunogenic tumors. Various antigenic preparations have been used to pulse DC in the past, including tumor cell lysates, acid-eluted peptides, synthetic tumor peptides, and tumor cell messenger or total RNA. Much debate exists as to which is the best pulsing approach to achieve optimal antitumor effects in vivo. The demonstration that DC pulsed with cell lysates obtained from one tumor cell line can be effective in inhibiting the growth of a second, histologically distinct tumor reaffirms the previous observation of shared tumor antigens among different tumors. Exploitation of this concept could potentially lead to advances in the treatment of poorly immunogenic human neoplasms.

REFERENCES

1. Egner W, Andreesen R, Hart D: Allostimulatory cells in fresh human blood. Transplantation 1993;56:945–950.
2. Nestle F, Zheng X, Thompson C, et al.: Characterization of dermal dendritic cells obtained from normal human skin reveals phenotypic and functionally distinct subsets. J Immunother 1993;151: 6535–6545.
3. Reid CD, Fryer PR, Clifford C, et al.: Identification of hematopoietic progenitors of macrophages and dendritic Langerhans cells (DL-CFU) in human bone marrow and peripheral blood. Blood 1990;76:1139–1149.
4. Steinman RM, Witmer MD: Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. Proc Natl Acad Sci USA 1978;75:5132–5136.
5. Levin D, Constant S, Pasqualini T, et al.: Role of dendritic cells in the priming of $CD4^+$ T lymphocytes to peptide antigen in vivo. J Immunol 1993;151:6742–6750.
6. Caux C, Massacrier C, Dezutter-Dambuyant C, et al.: Human dendritic Langerhans cells generated in vitro from $CD34^+$ progenitors can prime naïve $CD4^+$ T cells and process soluble antigen. J Immunol 1995;155:5427–5435.
7. Constant S, Sant'Angelo D, Pasqualini T, et al.: Peptide and protein antigens require distinct antigen-presenting cell subsets for the priming of $CD4^+$ T cells. J Immunol 1995;154:4915–4923.
8. Inaba K, Metlay JP, Crowley MT, Steinman RM: Dendritic cells pulsed with protein antigens in vitro can prime antigen-specific, MHC-restricted T cells in situ. J Exp Med 1990;172:631–640.
9. Huang AYC, Golumbek P, Ahmadzadeh M, et al.: The role of bone marrow-derived cells in presenting class I-restricted tumor antigens. Science 1994;264:961–965.
10. Porgador A, Gilboa E: Bone marrow-generated dendritic cells pulsed with a class I-restricted peptide are potent inducers of cytotoxic T-lymphocytes. J Exp Med 1995;182:255–260.
11. Steinman RM: The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 1991;9:271–296.
12. Aebersold P, Hyatt C, Johnson S, et al.: Lysis of autologous melanoma cells by tumor infiltrating lymphocytes: Association with clinical response. J Natl Cancer Inst 1991;83:932–937.
13. Teunissen MB, Wormmeester J, Krieg SR, et al.: Human epidermal Langerhans cells undergo profound morphologic and phenotypic changes during in vitro culture. J Invest Dermatol 1990;94: 166–173.
14. Simon JC, Cruz PD, Tigelaar RE, et al.: Adhesion molecules CD11a, CD18, and ICAM-1 on human epidermal Langerhans cells serve a functional role in the activation of alloreactive T-cells. J Invest Dermatol 1991;96:148–151.
15. Larsen CP, Ritchie SC, Pearson TC, et al.: Functional expression of the costimulatory molecule, B7/BB1, on murine dendritic cell population. J Exp Med 1992;176:1215–1220.
16. Flamand V, Sornasse T, Thielemans K, et al.: Murine dendritic cells pulsed in vitro with tumor antigen induce tumor resistance in vivo. Eur J Immunol 1994;24:605–610.
17. Inaba K, Inaba M, Witmer-Pack M, et al.: Expression of B7 costimulator molecules on mouse dendritic cells. Adv Exp Med Biol 1995;378:65–70.
18. Ohshima Y, Delespesse G: T cell-derived IL-4 and dendritic cell-derived IL-12 regulate the lymphokine-producing phenotype of alloantigen-primed naïve human CD4 T cells. J Immunol 1997; 158:629–636.
19. Heufler C, Koch F, Stanzl U, et al.: Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon gamma production by T helper 1 cells. Eur J Immunol 1996;26:659–668.
20. Koch F, Stanzl U, Jennewein P, et al.: High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. J Exp Med 1996;184:741–746.
21. Cella M, Scheidegger D, Palmer-Lehman K, et al.: Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. J Exp Med 1996;184:747–752.
22. Paglia P, Chiodoni C, Rodolfo M, Colombo MP: Murine dendritic cells loaded in vitro with soluble protein prime cytotoxic T lymphocytes against tumor antigen in vivo. J Exp Med 1996;183:317–322.
23. Porgador A, Snyder D, Gilboa E: Induction of antitumor immunity using bone marrow-generated dendritic cells. J Immunol 1996; 156:2918–2926.
24. Mayordomo JI, Zorina T, Storkus WJ, et al.: Bone marrow-derived dendritic cells serve as potent adjuvants for peptide-based antitumor vaccines. Stem Cells 1997;15:94–103.
25. Mayordomo JI, Zorina T, Storkus WJ, et al.: Bone marrow-derived dendritic cells pulsed with synthetic tumour peptides elicit protective and therapeutic antitumour immunity. Nature Med 1995;1:1297–1302.
26. Celluzzi CM, Mayordomo JI, Storkus WJ, et al.: Peptide-pulsed dendritic cells induce antigen-specific, CTL-mediated protective tumor immunity. J Exp Med 1996;183:283–287.
27. Shimizu J, Suda T, Yoshioka T, et al.: Induction of tumor-specific in vivo protective immunity by immunization with tumor antigen-pulsed antigen-presenting cells. J Immunol 1989;142:1053–1059.
28. Grabbe S, Bruvers S, Gallo RL, et al.: Tumor antigen presentation by murine epidermal cells. J Immunol 1991;146:3656–3661.
29. Boczkowski D, Nair S, Snyder D, et al.: Dendritic cells pulsed

with RNA are potent antigen-presenting cells in vitro and in vivo. J Exp Med 1996;184:465–472.
30. Grabbe S, Beissert S, Schwarz T, Granstein RD: Dendritic cells as initiators of tumor immune responses: a possible strategy for tumor immunotherapy? Immunol Today 1995;16:117–121.
31. Steinman RM: Dendritic cells and immune-based therapies. Exp Hematol 1996;24:859–862.
32. Inaba K, Inaba M, Romani N, et al.: Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J Exp Med 1992;176:1693–1702.
33. Szabolcs P, Avigan D, Gezelter S, et al.: Dendritic cells and macrophages can mature independently from a human bone marrow-derived post-colony-forming unit intermediate. Blood 1996;87: 4520–4530.
34. Bender A, Sapp M, Schuler G, et al.: Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. J Immunol Methods 1996;196:121–135.
35. Romani N, Gruner S, Brang D, et al.: Proliferating dendritic cell progenitors in human blood. J Exp Med 1994;180:83–93.
36. Caux C, Dezutter-Dambuyant C, Schmitt D, et al.: GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. Nature 1992;360:258–261.
37. Hsu FJ, Benike C, Fagnoni F, et al.: Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nature Med 1996;2:52–58.
38. Van den Eynde B, Lethe B, Van Pel A, et al.: The gene coding for a major tumor rejection antigen of tumor P815 is identical to the normal gene of syngeneic DBA/2 mice. J Exp Med 1991;173: 1373–1384.
39. Lethe B, van den Eynde B, van Pel A, et al.: Mouse tumor rejection antigens P815A and P815B: Two epitopes carried by a single peptide. Eur J Immunol 1992;22:2283–2288.
40. Topalian SL, Rivoltini L, Mancini M, et al.: Human $CD4^+$ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. Proc Natl Acad Sci USA 1994;91: 9461–9465.
41. Cox AL, Skipper J, Chen Y, et al.: Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science 1994;264:716–719.
42. Kawakami Y, Eliyahu S, Jennings C, et al.: Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. Proc Natl Acad Sci USA 1994;91:6458–6462.
43. Coulie PG, Brichard V, Van Pel A, et al.: A new gene encoding for a differentiation antigen recognized by autologous cytotoxic T lymphocytes on HLA-A2 melanomas. J Exp Med 1994;180:35–42.
44. Wang RF, Robbins PF, Kawakami Y, et al.: Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes [erratum published in J Exp Med 1995;181:1261]. J Exp Med 1995;181:799–804.
45. Traversari C, van der Bruggen P, Luescher IF, et al.: A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. J Exp Med 1992;176:1453–1457.
46. van der Bruggen P, Szikora JP, Boel P, et al.: Autologous cytolytic T lymphocytes recognize a MAGE-1 nonapeptide on melanomas expressing HLA-Cw* 1601. Eur J Immunol 1994;24:2134–2140.
47. Boel P, Wildmann C, Sensi ML, et al.: BAGE: A new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity 1995;2:167–175.
48. Van den Eynde B, Peeters O, De Backer O, et al.: A new family of genes encoding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J Exp Med 1995;182: 689–698.
49. Van den Eynde B, Gaugler B, van der Bruggen P, et al.: Human tumour antigens recognized by T cells: Perspective for new cancer vaccines. Biochem Soc Trans 1995;23:681–686.
50. Itoh T, Storkus WJ, Gorelik E, Lotze MT: Partial purification of murine tumor-associated peptide epitopes common to histologically distinct tumors, melanoma and sarcoma, that are presented by H-$2K^b$ molecules and recognized by $CD8^+$ tumor infiltrating lymphocytes. J Immunol 1994;153:1202–1215.
51. DeMatos P, Abdel-Wahab Z, Vervaert C, Seigler HF: Vaccination with dendritic cells inhibits the growth of hepatic metastases in B6 mice. Cell Immunol 1998 (in press).
52. Abdel-Wahab ZA, Osanto S, Darrow TL, et al.: Transduction of human melanoma cells with the gamma interferon gene enhances cellular immunity. Cancer Gene Ther 1994;1:171–179.
53. Abdel-Wahab Z, Dar M, Osanto S, et al.: Eradication of melanoma pulmonary metastases by immunotherapy with tumor cells engineered to secrete interleukin-2 or gamma interferon. Cancer Gene Ther 1997;4:33–41.
54. van Rensburg EJ, van Heerden WF, Raubenheimer EJ: Langerhans cells and human papillomaviruses in oesophageal and laryngeal carcinomas. In Vivo 1993;7:229–232.
55. Gallo O, Bianchi S, Giannini A, et al.: Correlations between histopathological and biological findings in nasopharyngeal carcinomas and its prognostic significance. Laryngoscope 1991;101:487–493.
56. Furihata M, Ohtsuki Y, Ido E, et al.: HLA-DR antigen- and S-100 protein-positive dendritic cells in esophageal squamous cell carcinoma—their distribution in relation to prognosis. Virchows Arch B Cell Pathol 1992;61:409–414.
57. Tsujitani S, Kakeji Y, Watanabe A, et al.: Infiltration of S-100 protein positive dendritic cells and peritoneal recurrence in gastric cancer. Int Surg 1992;77:238–241.
58. Tsujitani S, Kakeji Y, Maehara Y, et al.: Dendritic cells prevent lymph node metastases in patients with gastric cancer. In Vivo 1993;7;233–237.
59. Zeid NA, Muller HK: S100 positive dendritic cells in human lung tumors associated with cell differentiation and enhanced survival. Pathology 1993;25:338–343.
60. Bigotti G, Coli A, Castagnola D: Distribution of Langerhans cells and HLA class II molecules in prostatic carcinomas of different histopathological grade. Prostate 1991;19:73–87.
61. Nakano T, Oka K, Sugita T, Tsunemoto H: Antitumor activity of Langerhans cells in radiation therapy for cervical cancer and its modulation with SPG administration. In Vivo 1993;7:257–263.
62. Peoples GE, Goedegebuure PS, Smith R, et al.: Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-derived peptide. Proc Natl Acad Sci USA 1995;92: 432–436.
63. Yoshino I, Goedegebuure PS, Peoples GE, et al.: HER2/neu-derived peptides are shared antigens among human non-small cell lung cancer and ovarian cancer. Cancer Res 1994;54:3387–3390.
64. Peoples GE, Smith RC, Linehan DC, et al.: Shared T cell epitopes in epithelial tumors. Cell Immunol 1995;164:279–286.
65. Shamamian P, Kawakami Y, Restifo N, et al.: Recognition of neuroectodermal (NE) tumors by melanoma specific T-cells: Evidence for shared tumor associated antigens (Ag). Proc Am Assoc Cancer Res 1993;34:482.
66. Fidler IJ, Gersten DM, Hart IR: The biology of cancer invasion and metastasis. Adv Cancer Res 1978;28:149–250.
67. Nanni P, Colombo MP, De Giovanni C, et al.: Impaired H-2 expression in B16 melanoma variants. J Immunogenet 1983;10: 361–370.
68. Celik C, Lewis DA, Goldrosen MH: Demonstration of immunogenicity with the poorly immunogenic B16 melanoma. Cancer Res 1983;43:3507–3510.
69. Cohen EP, Kim TS: Neoplastic cells that express low levels of MHC class I determinants escape host immunity. Semin Cancer Biol 1994;5:419–428.
70. Bellone M, Iezzi G, Manfredi AA, et al.: In vitro priming of cytotoxic T lymphocytes against poorly immunogenic epitopes by engineered antigen-presenting cells. Eur J Immunol 1994;24: 2691–2698.
71. Bellone M, Iezzi G, Martin-Fontecha A, et al.: Rejection of a nonimmunogenic melanoma by vaccination with natural melanoma peptides on engineered antigen-presenting cells. J Immunol 1997;158:783–789.
72. Tanaka K, Gorelik E, Watanabe M, et al.: Rejection of B16 melanoma induced by expression of a transfected major histocompatibility complex class I gene. Mol Cell Biol 1988;8:1857–1861.

73. Wells AD, Rai SK, Salvato MS, et al.: Restoration of MHC class I surface expression and endogenous antigen expression by a molecular chaperone. Scand J Immunol 1997;45:605–612.
74. Bloom MB, Perry-Lalley D, Robbins PF, et al.: Identification of tyrosinase-related protein 2 as a tumor rejection antigen for the B16 melanoma. J Exp Med 1997;185:453–495.
75. Restifo NP, Esquivel F, Kawakami Y, et al.: Identification of human cancers deficient in antigen processing. J Exp Med 1993; 177:265–277.
76. Kaklamanis L, Leek R, Koukourakis M, et al.: Loss of transporter in antigen processing 1 transport protein and major histocompatibility complex class I molecules in metastatic versus primary breast cancer. Cancer Res 1995;55:5191–5194.
77. Weber JS, Jay G, Tanaka K, Rosenberg SA: Immunotherapy of a murine tumor with interleukin 2. Increased sensitivity after MHC class I gene transfection. J Exp Med 1987;166:1716–1733.
78. Chang AE, Shu SY, Chou T, et al.: Differences in the effects of host suppression on the adoptive immunotherapy of subcutaneous and visceral tumors. Cancer Res 1986;46:3426–3430.
79. Shu SY, Rosenberg SA: Adoptive immunotherapy of newly-induced murine sarcomas. Cancer Res 1985;45:1657–1662.
80. Spiess PJ, Yang JC, Rosenberg SA: In vivo antitumor activity of tumor-infiltrating lymphocytes expanded in recombinant interleukin-2. J Natl Cancer Inst 1987;79:1067–1075.
81. Chou TA, Chang AE, Shu SY: Generation of therapeutic T lymphocytes from tumor-bearing mice by in vitro sensitization: culture requirements and characterization of immunologic specificity. J Immunol 1988;140:2453–2461.
82. Dar MM, Abdel-Wahab Z, Vervaert CE, et al.: Immunologic memory induced by genetically transduced tumor cells. Ann Surg Oncol 1996;3:247–254.
83. Abdel-Wahab Z, Dar MM, Hester D, et al.: Effect of irradiation on cytokine production, MHC antigen expression, and vaccine potential of interleukin-2 and interferon-γ gene modified melanoma cells. Cell Immunol 1996;171:246–254.
84. Anichini A, Mortarini R, Maccalli C, et al.: Cytotoxic T cells directed to tumor antigens not expressed on normal melanocytes dominate HLA-A2. 1-restricted immune repertoire to melanoma. J Immunol 1996;156:208–217.
85. Nair SK, Boczkowski D, Snyder D Gilboa E: Antigen-presenting cells pulsed with unfractionated tumor-derived peptides are potent tumor vaccines. Eur J Immunol 1997;27:589–597.
86. Rosenberg SA, Yang JC, Schwartzentruber DJ, et al.: Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nature Med 1998;4:321–327.